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Cellular response to the ribonuclease injection; a morphologic and cytochemical study*

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Abstract

By the repeated injection of RNase into mice the histological, cytochemical and electronmicroscope observations of several tissues and the quantitative estimation of DNA contents per cell in liver have been conducted. The observations proved that the most marked changes occur in basophilia, ER (endoplasmic reticulum) and Palade's granules; the dissociation of the granules from ER and their agglomeration, and the final disappearance of the granules and ER. The increase of the granules in number surrounding the nucleus seen in liver cells and the appearance of the ring form ER in the pancreatic exocrine cells and its development from the nuclear membrane have been traced morphologically and these are comprehended as the regenerating picture of ER and granules from the nuclear outer membrane. DNA contents in liver cell increase in the early stage and decrease to the normal level in the later stage. The former is attributed to the cessation of mitosis by the damage of cell center without interference on DNA synthesis and the latter to the disappearance of the cells of tetraproidy by degeneration.

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CELLULAR RESPONSE TO THE RIBONUCLEASE INJECTION: A MORPHOLOGIC AND CYTOCHEMICAL STUDY

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The fact that ribonucleic acid (RNA) is essentially concerned in the protein synthesis has been definitely established by the work of Ochoa[1,2] and others (Caspersson[3], Brachet[4,5,6,7,8,9] etc.). Quite recently, Brachet and Chévremont and others reported the interesting findings which show the penetration of RNase into living cells, the observations on amaebae, cultured fibroblasts, blood cells, and cancer cells (Brachet,[4,5] Chévremont,[10,11] Kaufman[12], Das[13], Ledoux[14,15], etc.). The experiments proved that the RNase penetrated into living cells interferes with the RNA metabolism and thus brings about inhibition of mitosis, abnormal cell division and degeneration of the cells. This fact seems to give a clue to clarify the unknown metabolic pathway of RNA in living cell. At present, in living cell RNase is believed to be contained in the mitochondria or lysosomes (De Duve, B. C. Pressman, R. Wattrianx, etc.)[16], but the questions why RNase is contained so aboundantly in the cell and how this enzyme is associated with the metabolism of RNA are problems for future study. With the purpose to solve these problems we have studied the changes occurring in the living cells after the intravenous injection of RNase into animals morphologically as well as cytochemically, using RNase from bovine pancreas whose chemical structure and the acting mechanism on RNA are recently clarified[17,18,19,20,,21,22,23,24,25,26]. In this paper the morphologic changes of several organs and the changes in submicroscopic structure of the cells of pancreas and liver and the changes in DNA contents in liver cells after RNase injection are reported.

MATERIALS AND METHODS

The RNase used in the present experiment was obtained from the bovine pancreas according to the McDonald's method[22], i. e. one gram crystal substance containing RNase was obtained by extracting 4 liters

bovine pancreas brei with ammonium sulfate in a chilled room (0°C—4°C). This crystalline substance was proved to have the ability to remove completely the cytoplasmic basophilia of the A. H. 130 cell by exposing the smeared and fixed cells to 0.05% RNase solution, pH 7.4, for 60 minutes at 50°C, as revealed by the post-staining with pyronine methylgreen or Giemsa.

As the experimental animals 40 normal hybrid mice were used dividing into 5 groups, 8 animals each. In each group 4 animals were treated with RNase solution, another three were injected with the buffer solution containing no RNase and the last one was left without any treatment. The RNase solution for the injection was prepared by solving 50 mg to 100 mg of RNase into 10cc. of the phosphate buffer solution prepared with 0.066 M-Na_2HPO_4 and 0.066 M-KH_2PO_4 pH 7.4. The solution was prepared freshly just before the injections. The injections of RNase and the buffer solution were carried out intravenously daily for the first 4 days, 0.3 ml per animal. Thereafter, for ten successive days 0.4 to 1 ml of the solution was injected intraperitoneally per day, regulating the amount of injection according to the general condition of animals. And then for the duration of 14 days to 31 days thereon the injections were continued every day or every other day until the total dosage of RNase reached 350 or 600 mg in each animal treated. Observations were carried out at 4th, 6th, 14th, 16th, and 31st day of the experiment.

The several fragments of organs were fixed in Carnoy's fluid, alcohol, Orth, and 10% formalin solution respectively, and after embedding into paraffin the sections were made. These sections were observed after the staining with pyronine methylgreen (Brachet), PAS stain (McManus), and glycogen stain (Best's-carmine). Besides these, small fresh tissue fragments were fixed in 1% osmic acid solution for one hour and their sections were observed by electron microscope. Peripheral blood, bone marrow, spleen, liver, and kidney were imprinted and stained with Giemsa for the cytologic observation. The quantitative determination of DNA per cell was carried out at 5,600 Å by the method of Swift using the spectromicrophotometer of the Olympus model on the liver cells in paraffin sections stained with Feulgen reaction by the method of Shibatani. The DNA contents are measured in 50 cells in each sample.

RESULTS

Morphological and Cytochemical Findings : Among the tissues examined the most striking changes are found in the liver. After 14 to 20 days' treatment with the RNase an enlargement of the nuclei and the swel-

ling of cytoplasm can be observed, and a considerable number of liver cells with two nuclei appeared mainly in the central part of acini. A group of the liver cells lying at the peripheral area of the lobules is unevenly compressed and becomes atrophied, reduced in size (Plate 1). Consequently, the picture resembles that of the lobular hypertrophy as in liver cirrhosis, but shows no sign of reconstruction of the tissue. The noteworthy finding is the marked increase in cytoplasmic basophilia around the nuclei in the large swollen liver cells situated in the central area of acini. The basophilia appears granular in some cells and diffuse in others (Plates 2, 3). The basophilic area around the nucleus is stained deep red by pyronine methyl-green staining. Some of these liver cells show vacuolization in their cytoplasm (Plate 3). The changes occurring in the cytoplasm become severe along with the increase in number of injections or the increase in the amount of RNase injected ; a high degree of degeneration and devastation, for example, in the animals observed after the RNase injection of 600 mg in total dosage (Plate 4).

After 2 to 3 weeks' treatment in some cells there can be demonstrated a marked decrease in glycogen contents, especially in those cells swollen and enlarged and found to be lying in the central part of acini but the cells lying in the peripheral layer of acini are rich in PAS positive granules (Plates 5, 6). A marked irregularity in glycogen contents can be seen. But on the whole, it is to be noted that the liver glycogen is slightly decreased in the animals treated with RNase in comparison with that of the control. Observations on the liver of the animals treated with RNase for the longest period (30 days), injected 600 mg of RNase as total, the PAS positive granules are found to be rich and no actual difference from those in control animals, suggesting that the slight decrease at the initial stage will be recovered back to the normal level with the increased resistance of the animal to RNase.

In the kidneys no striking changes can be observed in any specimens. Only in some cases treated with RNase for a long period of time a slight degeneration of the epithelium of convoluted tubules can be recognized.

In the pancreas the initial changes are not so marked, but after a repeated treatment with RNase for a long period the degenerative changes of exocrine cells can often be observed.

The pancreas tissues show a localized distribution of the area, where the cytoplasimc basophilia and the number of zymogen granules, are remarkably reduced (Plate 7). In two cases out of the entire group examined the swelling of Langerhan's island has been recognized (Plate 8).

The digestive tracts themselves show hardly any morphological change

comparing to those of the control animals, but the lymph apparatus found in the digestive wall was found to be enlarged with the proliferation of reticulum cells and lymphcytes and in one case the stenosis of the intestine was recognized by the enlarged lymph nodes.

The cells of central nervous system are generally not affected so markedly by the treatment, but some cells, Purkinje cells in particular, show a degenerative change, necrobiosis or a marked deposition of brown pigment, and the decrease in their stainability in PAS staining.

Besides these, some parts of the muscle layer of the peritoneal wall which correspond to the way of RNase introduction at the injection show a severe degeneration, especially marked in the case given the repeated injection.

The findings on the cells in the imprinted specimens actually show no difference from those of paraffin sections, but in these specimens it has been clearly demonstrated that the cells in mitosis is extremely reduced in number comparing to those from the control animals.

Electron microscopic findings : Electron microscope observations have been carried out only on the liver parenchymal cells, Langerhan's island cells and pancreatic exocrine cells which showed the most striking changes by light microscope.

The liver cells showed a striking change under electron microscope, too, i. e. the aggregation of cytoplasmic granules (Palade's granules) followed by the appearance of scanty area in the perinuclear zone. The changes in the early stage are the discharge of the granules from endoplasmic reticulum (ER) and a marked agglutination of the discharged granules mainly in the perinuclear area, which appear most strikingly in liver parenchymal cell. The discharge and agglutination of the granules differ in degree from cell to cell, but the picture shows that the basophilia in the perinuclear area as observed by light microscope corresponding to the agglomerated masses of the discharged granules.

Further continual injection of RNase makes the aggregated granules reduced in number (Plates 9, 10, 11) and some agglomerated granules are found to be arranged in a radial formation on the nuclear membrane (Plate 12) and at last they disappear completely probably by the advanced digestion with RNase, leaving only radially arranged transparent vacuoles around the nucleus (Plate 11). In the perinuclear area where the agglomerated mass of granules or vacuolated structure can be seen, swollen mitochondria can often be observed in radial arrangement to the nucleus, too.

Besides these, in a certain part of the cytoplasm the ER which looks

nearly normal or is arranged in a lined-up style can be observed mainly being situated around mitochondria (Plates 10, 12). The area appearing transparent with some vacuolated structure under electron microscope is in all likelihood identical with the transparent layer observable in light microscopy. Outer nuclear membrane of liver parenchymal cell also carries Palade's granules. These granules are also discharged and disappear by the continued treatment with RNase as in the case of those of ER in cytoplasm.

In the pancreatic exocrine cells a marked irregularity can be observed in the degree of damage from cell to cell, as suggested from the light microscope findings. Some cells are nearly normal and others have the extremely-deranged ER's and granules with partial indistinctness of the membrane and the discharge of granules (Plates 13, 14, 15). The nuclear membranes are also disintegrated (Plates 13, 14). Zymogen granules are markedly reduced in number and in size suggesting a marked inhibition of protein synthesis. Some of the pancreatic exocrine cells, as shown in Plates 16 and 17 have ring-like ER's of double membrane, which are distributed densely. The picture shown in Plate 16 clearly indicates that the ER can be formed by the shoot-like growth of the nuclear membrane and the ring-like structures seem to have been formed by an indentation of a part of ER, or appear by the transverse section of the sprouted ER, into the coved adjacent ER as Palade's granules are observed both on outside and inside as well.

Some cells of the Langerhan's island showed also a marked changes. In the cells shown in Plate 18 there are a considerable number of the secretion granules situated in the round ER. The round vacuoles will be formed by the swelling of the double membrane of ER but no formation of the secretion granules. But just near these cells there are found also the cells which contain only a small number of secretion granules and less opaque in cytoplasm and have striated ER (Plate 19). The latter will be the regenerated ER, because they are rich in Palade's granules and contain no secretion granules.

Changes in the DNA contents per cell: The changes in the DNA contents as observed by the microspectrophotometer at the intervals of 14, 16, and 30 days after the intraperitoneal injection of RNase demonstrated that by the 14 days' treatment there appear a number of cells having a markedly high DNA contents equivalent to the tetraploidy, but 16 to 30 days' treatment reduced the DNA contents, all the cells being replaced with those having DNA equivalent to diploid. (Fig. 1)

The similar changes to those found in the animals treated with RNase

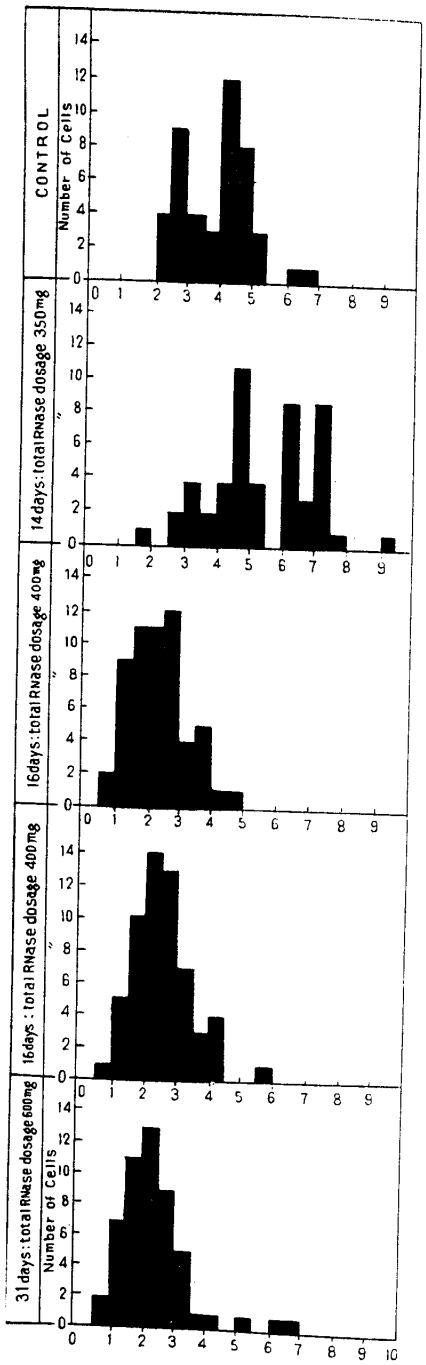

Fig. 1. Diagrammatical demonstration of distribution of the liver cells according to their DNA contents

DNA contents have been measured on the sections with Feulgen reaction. In each observation, 50 cells have been observed.

have not been observed in both the control animals fed on the same diet and those injected with buffer solution containing no RNase.

DISCUSSION

The results demonstrated above clearly show that the RNase introduced into living body reaches the various organs through the circulating system and causes the specific changes on the cells whose characteristics are very similar to those found in the cells exposed to RNase *in vitro* as revealed by KAUFMANN[12], DAS[13], Brachet[4,5,6], Chévremont[10,11] *et al.* (1957). The most susceptible organ to the injection of RNase is liver and in a less extent the pancreas, and kidney and some nerve cells are also affected to a slight degree. The striking change characteristic to the RNase treatment is a reduction in cytoplasmic basophilia followed by the degeneration of cells. The changes in liver cells show a marked difference between those lying in the acinar center and those in peripheral areas as already described. This may be due to the difference in the amount of RNase penetrating into cells for a certain period, as the streaming velocity of blood, in which RNase is contained, is supposed to be higher in the central part of acini than in the periphery, or due to the difference in the function of the cells between those lying in the center and periphery. The true reason is obscure at present.

Electron microscopy has revealed that the increase in basophilicity around the nucleus is presented by the agglomeration of Palade's granules especially marked around the nucleus and the loss of basophilicity by the further-durated treatment is caused by the disappearance of the granules. The discharge of the granules from ER and their final disintegration will be due to the direct action of RNase on the granules, because in the fractionated microsomes it has been proved they lose their RNA by treating with RNase, as revealed by Palade and Siekevitz[23] and the observations by Seno and Yoshizawa[24] on frozen-dried cells revealed that Palade's granules disappear by exposing the sections to RNase, demonstrating that Palade's granules are actually the carrier of RNA. Observation revealed that Palade's granules agglomerating around the nucleus remain when the other part of the granular agglomeration disappear. This may show some regeneration of granules from the nuclear outer membrane as will be discussed precisely later on pancreatic exocrine cells.

Pancreatic exocrine cells are not so sensitive to the injection of RNase but in the later stage it has been also demonstrated that granules are discharged from ER and the outline of the ER becomes ambiguous and

some cells show degeneration or disintegration as in the case of liver parenchymal cells.

Palade and Siekevitz[23] (1956), recognized that the microsomes from pancreas are more labile than those from liver, but both of them are affected similarly by RNase. Severer change in liver than that in pancreas seen after the injection of RNase as observed in our experiment will be due to the difference in the amount of RNase reaching these organs or to the difference in the permeability of the cell membrane to RNase.

Besides these changes showing the degradation of organellae, the pictures suggest the regeneration process of ER and the granules. As pointed out previously, some pancreatic exocrine cells have a number of ER's in the ring form of various size or lined up with narrow spacing. They are irregular in distribution but they will be the newly formed ER's as they have Palade's granules and distinct in their wall and different from those disintegrating as described above. The nuclear membrane also shows a similar changes in degeneration as in ER but in some cells the double membrane structure can be seen clearly and suggests the development of ER from the outer nuclear membrane. In Fig. 2 of diagrammatical drawing

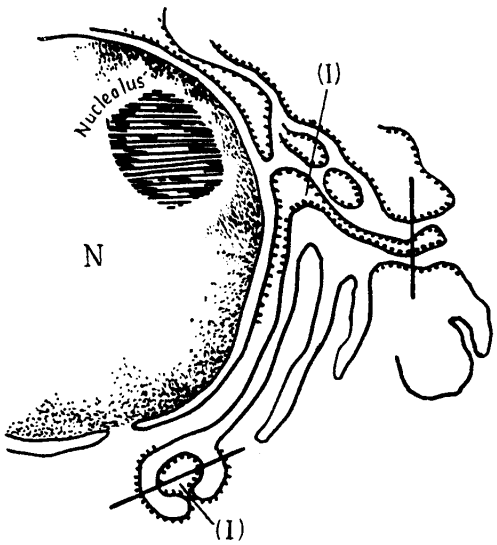

Fig. 2. Diagrammatical drawing of the ER formation.
In Fig. 2 a diagrammatical drawing of the formation of ER from the nuclear membrane according to the picture shown in Plate 17 is presented. The outer membrane forms a rod-like shoot and some indentation (I) occurs at the top of the shoot. Densely developed ER's are flattened by compressing each other and the lamellar structure as observed by Porter may be formed. The ring form structure will be seen at the external surface shown with two heavy lines and shows the newly formed ER's. N: Nucleus.

ing of the formation of ER from nuclear membrane drawn according to the picture shown in Plate 16 is presented. The outer membrane will form a rod-like shoot at first and some indentation may occur at the top of the shoot.

Densely developed ER will be flattened by compressing each other and the lamellar structure as observed by Porter may be formed. The ring form structure will be seen at the cut surface shown with two heavy lines in the picture and shows the newly formed ER.

Further observation will be required to decide persistent development of the ER from the nuclear outer membrane but there is a great possibility that in regeneration ER's develop as a rod-like shoot from the nuclear membrane and the indentation occurs from their top, which will appear as the ring-form ER in the profile, and due to the increase in population they develop to the lamellar form by being compressed with each other.

Caspersson[3] observed the motor nerve cells and recognized an increase in basophilia around the nucleus in the stage of restoration from the loss of RNA which can be induced by the exhaustive contraction of muscle belonging to the motor cells and he claimed that the production of RNA begins at the perinuclear area in the stage of restoration. He did not observe the change by electron microscope, but there is a possibility that the increase in basophilia around the nucleus presents the picture of regeneration of ER and the granules around the nuclei, as suggested above. The double layer structure of the nuclear membrane is demonstrated to remain in the increaseing stage of perinuclear basophilia and the outer membrane may be correlated to the reproduction of ER and the granules situated at the outer nuclear membranes carry the Palade's granules. Severe damage by RNase makes the granules around the nucleus disappear and in this stage the double layer structure of nuclear membrane is hardly visible. This finding supports the view that the nuclear outer membrane is responsible for the formation of new ER's and the granules, and the destruction of the nuclear membrane gives rise to the cessation of the production of them.

In the cells of Lanerghan's islet there can be seen abundant secretion granules surrounded by the membrane, demonstrating the picture of the production of the secretion granules by ER comparable to the picture shown in the pancreatic exocrine cells by Palade and Siekevitz[23]. In our case the ER membrane becomes ambiguous in treating with RNase but in some cells whose cytoplasm is less opaque and less in secretion granules, there appear the striated structures of ER. They are rich in Palade's granules

and probably the newly formed ER. They will become round when the secretion is initiated.

The large liver cells appearing in the acinar center after 14 days' treatment with RNase show the increase in DNA contents which roughly correspond to the DNA contents in tetraploid cell. This will mean the cessation of the cell division without the cessation of DNA synthesis. As is known, the cell center, which is the promoting center of the cell division, has RNA and it is supposed that RNase penetrates into nucleus and stops the activity of the cell center, by which the cells on mitosis stop the division but the DNA synthesis proceeds as usual and the appearance of tetraploid large cells is the result.

In vitro experiments done by several authors demonstrated that the cells exposed to RNase often cause abnormality in cell division. For instance, Chèvremont has found that the fibroblast exposed to RNase in culture stops the mitosis completely but the DNA synthesis proceeds almost normally. He attributed this effect of RNase to the destruction of centrosomes. Kaufman and Das also have found the same phenomena on onion root tip treated with RNase, revealing marked changes in the nucleus and asters. Brachet also demonstrated the mitotic abnormality in sea urchin embryo in blastula stage by exposing it to RNase temporarily.

The reduced DNA contents to the diploid level seen in the later stage of treatment will be due to the degeneration and the disappearance of tetraploid cells, because the cell degeneration proceeds on these large cells with the vacuolization of cytoplasm as revealed by electron microscope.

Thus it has been demonstrated that RNase introduced into the living body acts as to cause a severe cell damage which is primarily concerned with the change in the organellae having RNA and comparable to that seen by several authors on the cells exposed to RNase *in vitro*.

SUMMARY

By the repeated injection of RNase into mice the histological, cytochemical and electronmicroscope observations of several tissues and the quantitative estimation of DNA contents per cell in liver have been conducted.

The observations proved that the most marked changes occur in basophilia, ER (endoplasmic reticulum) and Palade's granules; the dissociation of the granules from ER and their agglomeration, and the final disappearance of the granules and ER.

The increase of the granules in number surrounding the nucleus seen in liver cells and the appearance of the ring form ER in the pancreatic ex-

ocrine cells and its development from the nuclear membrane have been traced morphologically and these are comprehended as the regenerating picture of ER and granules from the nuclear outer membrane.

DNA contents in liver cell increase in the early stage and decrease to the normal level in the later stage. The former is attributed to the cessation of mitosis by the damage of cell center without interference on DNA synthesis and the latter to the disappearance of the cells of tetraproidy by degeneration.

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Plates

Plate. 1

Fig. 1. The Mouse liver given intraperitoneal injection of RNase, the total dosage of 350 mg. Sacrificed 14th days after the start of the injection. Orth fixation, H-E stain.

In the upper portion there can be seen a lobule possessing swollen liver cells, in which the nuclei are swollen and basophilia appear in the perinuclear zone (A ↑). DNA contents in the swollen cells estimated by a microspectoptometer on another sections from the same sample proved the increase in DNA equivalent to tetraploidy. The lower portion is occupied by the atrophied cells probably by the compression. (B ↑)

Fig 2. An enlarged picture of the swollen cell appearing in Fig. 1. This cell presents the granules of basophilia (arrows) in the perinuclear area with the deeply stained nucleus.

Fig. 3. Swollen mouse liver cells seen in a sample from the mouse treated with RNase, total dosage, 350 mg. (on the 14th day). Carnoy's fixation, pyronine methyl green stain. The perinuclear area is stained deeply by pyronine (arrows), but the cytoplasm is rather vacuolated probably by the advanced degeneration. In the lower portion there are appearing some compressed cells with picnotic nuclei.

Fig. 4. The Liver from the mouse treated with RNase, total dosage of 600 mg. Sacrificed on the 31st day. Orth fixation, H-E stain. The cytoplasm is swollen and vacuolated. The basophilia are remarkablly reduced with the pycnosis of nuclei.

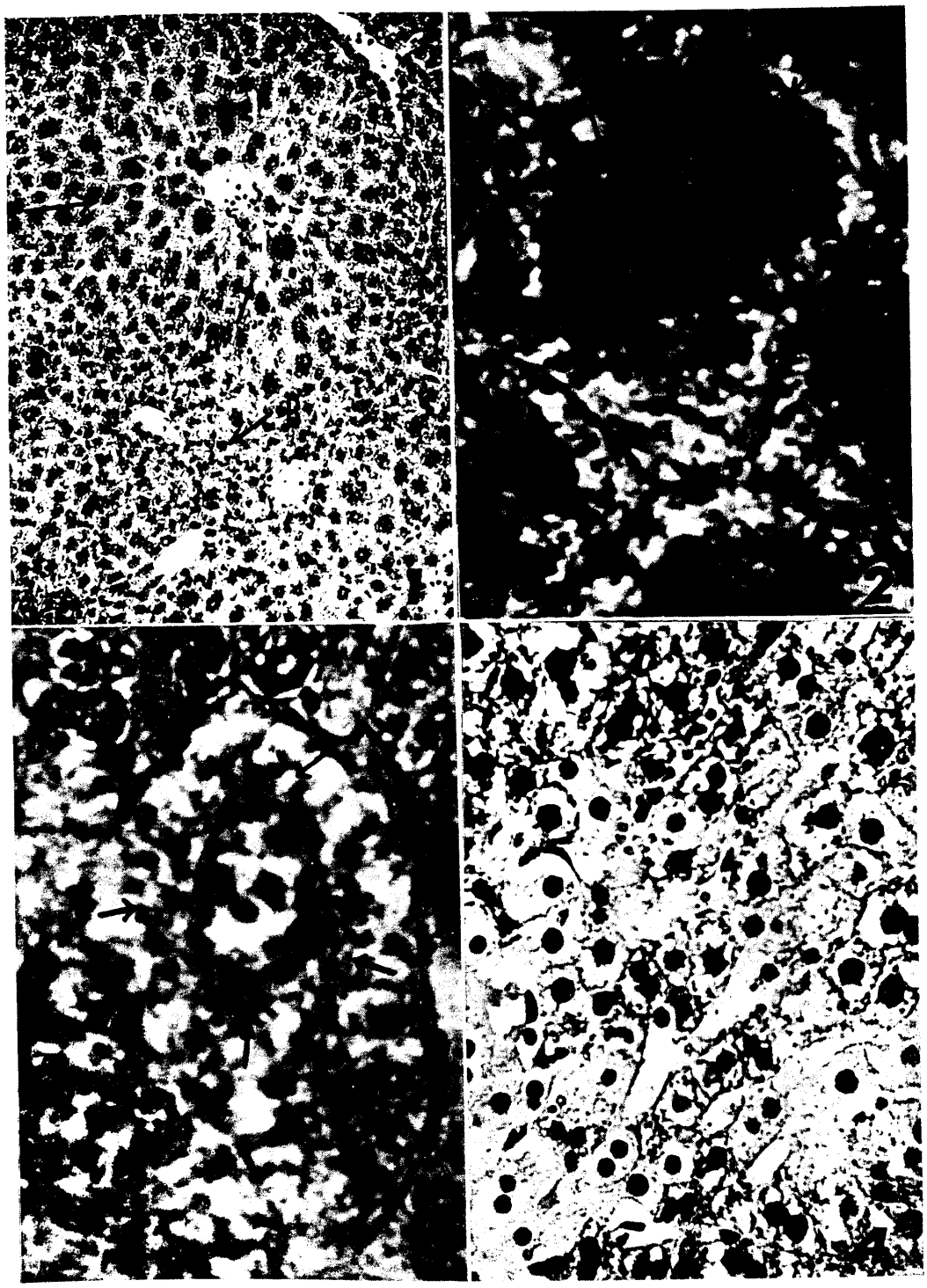

Plate. 2

Fig. 5. The liver of the mouse treated with RNase, total dosage 290 mg. Sacrificed on the 16th day. Alcohol fixation, PAS stain. The swollen cells lying in the central part of acini appear scanty in PAS positive granules. The peripheral area of acini gives a strongly positive reaction. (arrows)

Fig. 6. The mouse liver. An enlarged picture of a part of the same liver appearing in Fig. 5. Alcohol fixation, PAS stain. PAS positive granules can be seen in cytoplasm (A ↑) but some liver cells show a decrease in glycoprotein (B ↑). A marked irregularity in glycogen contents from cell to cell.

Fig. 7. The mouse pancreas treated with RNase, total dosage 600 mg. Killed on 31st experimental day. Orth fixation and H-E stain. Picture shows the exocrine cells and a marked irregularity in the basophilicity of cytoplasm from cell to cell and some cells lost completely their basophilia (arrows).

Fig. 8. The picture of Langerhan's islet of the mouse pancreas treated with RNase, total dosage of 290 mg. Sacrificed on 16th day. Orth fixation, H-E stain. Picture shows the swelling of the cells of Langerhan's islet with some pycnotic nuclei.

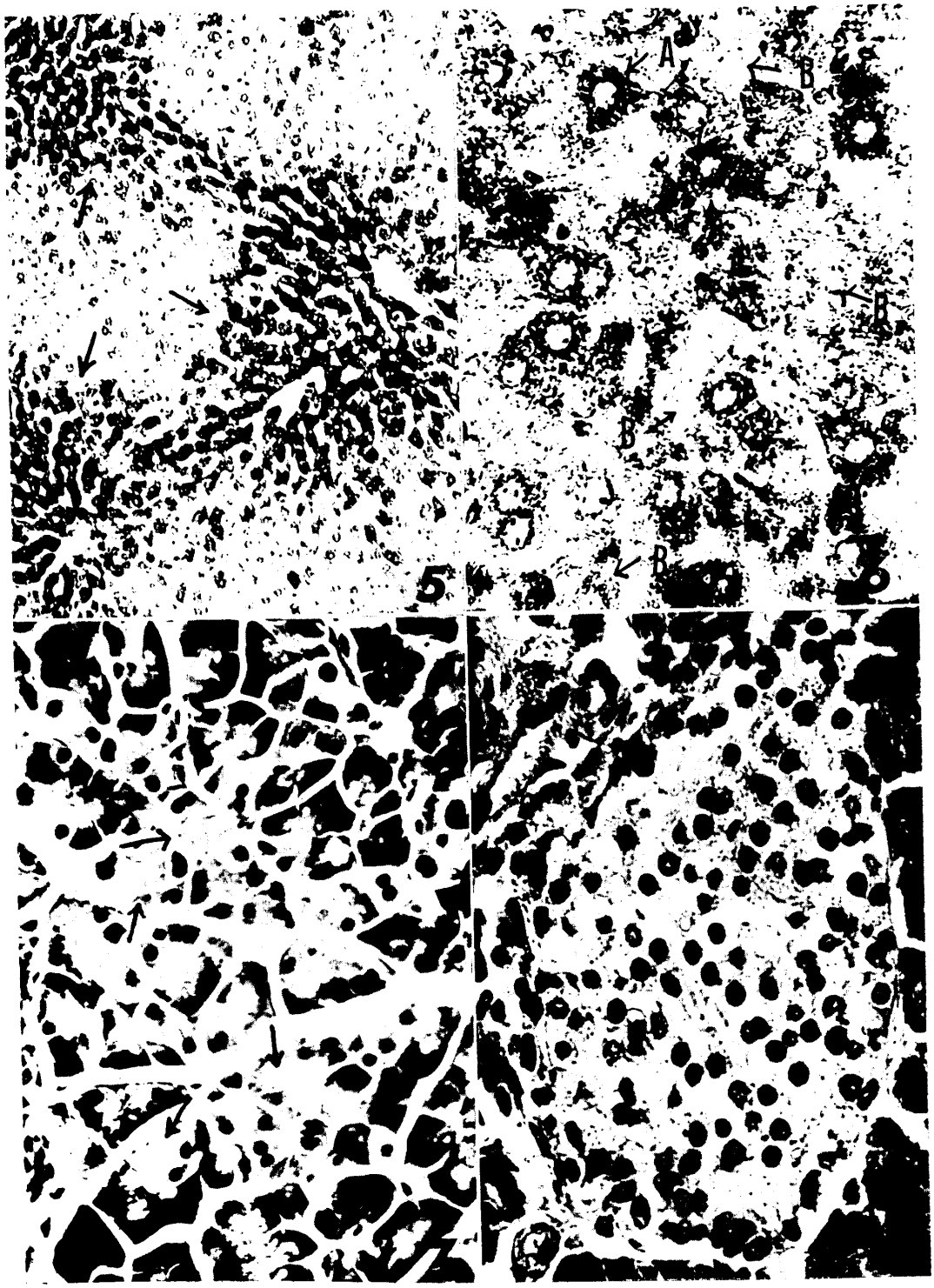
A
B
B
B
B
5
3

Plate. 3

Fig. 9. The cytoplasm of a liver parenchymal cell of the mouse treated with RNase, total dosage of 350 mg. Sacrificed on 14th day. The agglomeration of small dense particles can be seen, but no distinct structure of ER (arrows). The double membrane structure of some endoplasmic reticulum (ER) becomes ambiguous in a part. Mitochondria show destruction of their double membrane structure (M). N: Nucleus. G: Granulues of RNA particles

Fig. 10. A liver parenchymal cell of the mouse treated with RNase, total dosage of 400 mg. Killed on the 16th day. Cytoplasm appears clear and reduced in ER. Small dense or opaque agglutinated masses of the granules can be seen (arrows). Some of mitochondria show the indistinctness in their double memhrane structure (M). ER's are found to be gathered forming a striated mass lying densely around the mitochondria. N: Nucleus.

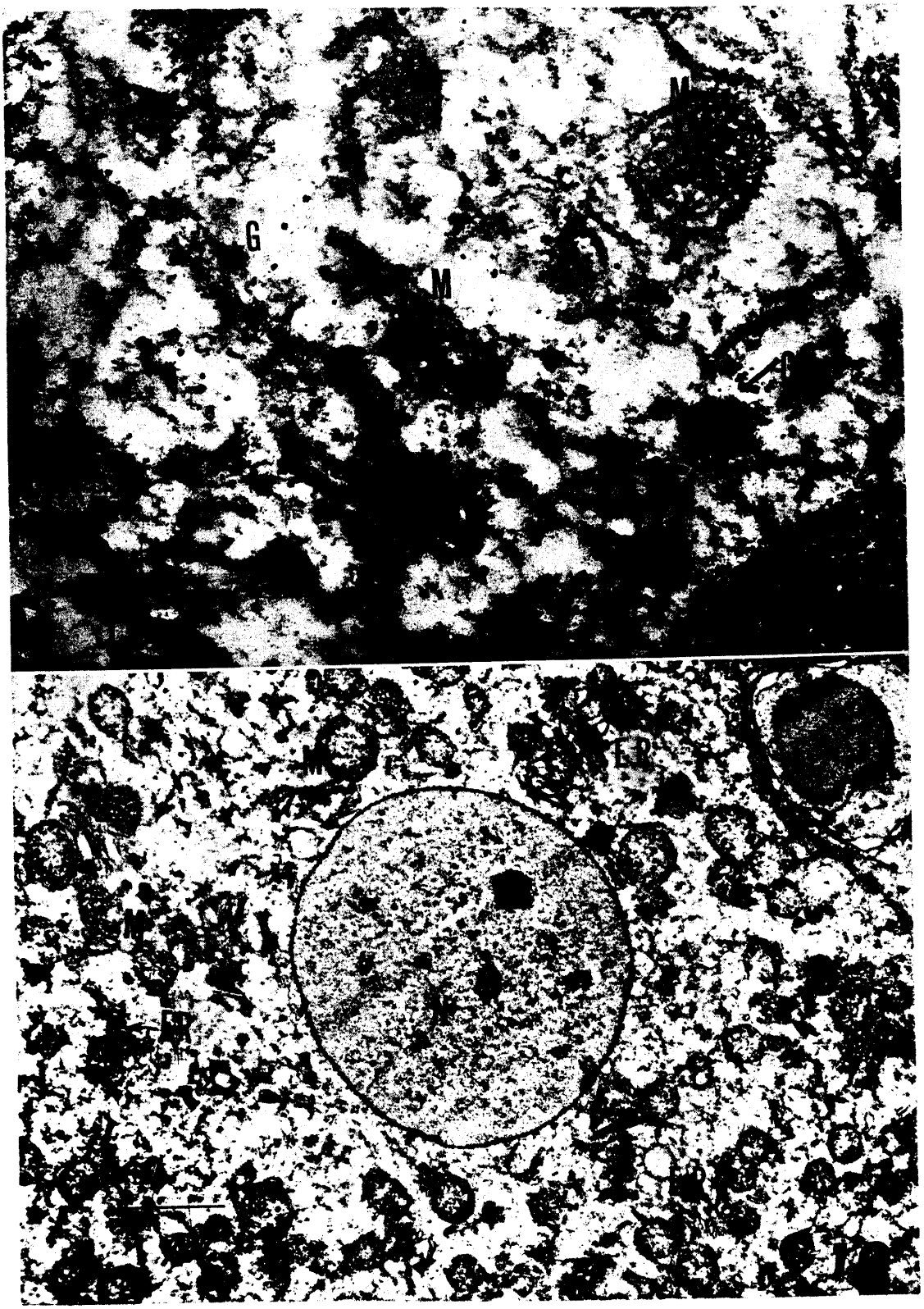
G
M
M
M
ER

Plate. 4

Fig. 11. A cell of the mouse liver treated with RNase, total dosage of 350 mg. Killed on 14th day. Electron dense granules can be seen surrounding the nucleus, which are agglomerated in the radial arrangement (G). In the cytoplasm also the agglutinated masses of the granules are seen. Nucleus (N), which shows some degenerative change with some agglutination of the contents. Small dense granules (D) are probably of lipids. M: Mitochondria.

Fig. 12. A liver cell of the mouse treated with RNase, total dosage of 350 mg. Killed on 14th day. Vacuolated ER's free of granules can be seen. These are arranged radial to the nuclear membrane (G) and mitochondria (M). Densely gathered ER's of clear double membrane are found around the mitochondria. N: Nucleus.

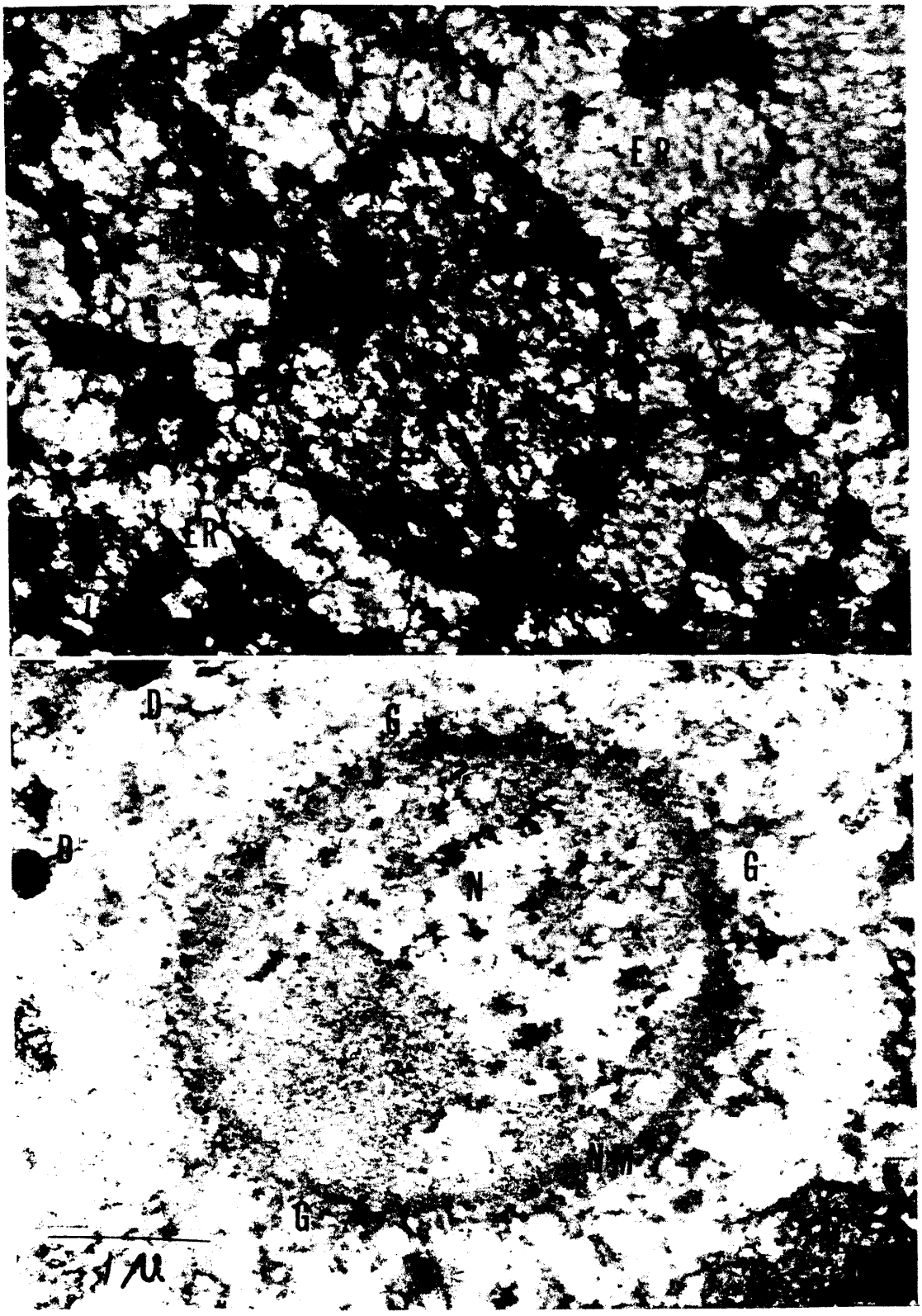
ER
ER
D
G
D
G
N
G

Plate. 5

Fig. 13. A part of a pancreatic exocrine cell of the mouse treated with RNase, total dosage of 600 mg. Killed on 31 st day. A number of Palade's granules (G) free of ER and agglomerated, can be seen in cytoplasm. The membrane of ER becomes ambiguous here and there (ER) and disappear partially. A part of mitochondrial membrane becomes ambiguous. The nuclear membrane (NM) becomes ambiguous. N: Nucleus. Z: Zymogen granule, M: Mitochondria

Fig. 14. A part of an exocrine cell from the pancreas of the mouse treated with RNase, total dosage of 600 mg. Killed on 36th day. The picture shows a complete disintegration of the nuclear membrane (NM) and the vacuolated swelling of ER (ER) with indistinctness in the contrast to the granules. (G)

Fig. 15. A part of cytoplasm of the pancreatic exocrine cell of the mouse treated with RNase, total dosage 600mg. Killed on 31 st day. In this cell a marked indistinctness of the ER membrane (right upper area), the swelling (lower central area) the discharge of the granules (G) and the agglomeration can be seen, but in some part the round and lined-up ER's appear.


N
E-R
N-M
G
73
74

Plate. 6

Fig. 16. A pancreatic exocrine cell from the mouse treated with RNase, total dosage of 600 mg. Killed on 31st day of experiment. In this cell there appear very clear structures of ER though they are peculiar in shape. The nuclear membrane (NM) is retained clearly and in one part the continuity of the outer nuclear membrane with the ER can be seen. Picture shows the formation of ER from the nuclear membrane which is elongating as a shoot into cytoplasm. Round and elongated structures of ER show that they are formed by the compression or the indentation of the sprouting nuclear membrane. NS: Nucleolus.

Fig. 17. A part of a pancreatic exocrine cell of the mouse treated with RNase, total dosage of 600 mg. Killed on 31st day. In this cell a marked irregularity in the shape of ER. Almost all of them appear round in section, but again a shoot-like formation of ER on the nuclear membrane can be seen (S). A swollen mitochondrion can be seen on the right middle part of the picture (M). NH: nuclear membrane


M
ER
M
ER
ER
M
16
17

Plate. 7

Fig. 18. Cell of Langerhan's islet from the pancreas of the mouse treated with RNase, total dosage 600 mg. Killed on 31st day. Small dense secretion granules (Z) can be seen abundantly but the double membrane picture of ER is hardly visible. Some of these secretion granules are surrounded by the membrane (ER) but some of them are indistinct in their surrounding membrane. Besides these, some large vacuolated structures appear (V). The space of the double nuclear membrane is enlarged in some parts (NM).

Fig. 19. A cell from the same specimen as No. 18. ER granules surrounding each secretion granule are indistinct (Z) but in the lower part there can be seen the striated ER (ER) of double membrane having Palade's granules (G). This area lacks in the secretion granules and the ER's will probablly be the newly formed ones.


18
19